The effect of oral zinc supplementation on bone biomarkers in rats in the experimental model of space exploration (simulated weightlessness)

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Research Article

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Abstract

Background: Zinc as a cofactor is effective in the function of many enzymes and is also essential for collagen synthesis and bone mineralization.

Objectives: The current study aims to survey the effect of oral zinc supplementation on bone biomarkers (alkaline phosphatase and osteocalcin) in rats in the experimental model of space exploration (simulated weightlessness).

Methods: This was an experimental study. A laboratory model of space exploration for inducing weightlessness in rats was done by the Hindlimb suspension technic. Rats were randomly divided into 2 groups; group A (n=7) received daily zinc sulfate and group B (n=7) received daily placebo. Rats received the supplement or placebo for 30 days. The alkaline phosphatase and osteocalcin were assessed in both groups at baseline and 30 days.

Results: The microgravity simulation model of space exploration increased significantly alkaline phosphatase and decreased significantly osteocalcin in rats. Adding zinc sulfate to the diet has been effective in increasing the amount of alkaline phosphatase but has not shown a significant effect on the amount of osteocalcin reduction.

Conclusion: According to current findings, it seems that Zinc administration did not have an effect on osteocalcin improvement but led to more rising of alkaline phosphatase in rats.

Introduction

The Human Research Roadmap (HRR) has been published by NASA to systematically target bio-aerospace research to reduce or eliminate risks to health, safety, and performance during and after space exploration. Risks include physiological and performance effects from hazards such as radiation, altered gravity, and hostile environments, as well as unique challenges in medical support, human factors, and behavioral health support. Of the 23 risks identified in this roadmap for humans in space, at least 5 are associated with changes in the function of the musculoskeletal system. The main concerns are about the increase in age-related osteoporosis, the lack of improvement in bone loss after returning from space exploration, and the increased risk of fractures (1).

It is now well established that weightlessness during space exploration causes osteoporosis. Loss of bone mass throughout the body and in the legs, which are rich in cortical bone, is 0.3–0.4% per month. The reduction in bone mass in the trabecular part of the bone seems to be greater than in the cortical part (2, 3).

Histomorphometric studies of rats that experienced space exploration for different periods showed changes in bone cortex and sponge, a transient increase in bone resorption, and a permanent decrease in bone production (4, 5).

Data on interventions to maintain stable bone metabolism and prevent bone loss during space exploration are limited. Ingesting high amounts of calcium and vitamin D supplements during space exploration did not prevent osteoporosis. The additives did not prevent an increase in bone resorption although they did prevent an increase in serum calcium levels (6, 7).

Zinc is an essential element that has many physiological roles in biochemical processes, especially growth in humans and animals. Several zinc-dependent hormones and enzymes are involved in bone metabolism. Zinc stimulates cell differentiation, cell proliferation, and mineralization in osteoblasts and thus stimulates bone production. From a molecular point of view, zinc stimulates gene expression in various proteins including Runx2/Cbfa 1 (transcription factor for osteoblast cell differentiation), type I collagen, alkaline phosphatase, and osteocalcin in cells (8). The concentration of osteocalcin, which is secreted from osteoblastic cells in the culture medium, rises significantly after the addition of zinc. Adding zinc to culture stimulates the production of bone growth factors and bone matrix proteins that are involved in promoting bone formation and mineralization. DNA polymerase enzyme involved in DNA production is a zinc-dependent enzyme. Zinc may cause DNA production by activating the DNA polymerase enzyme in bone tissue osteoblasts (9).

Bone biomarkers are used for research as well as to monitor the therapeutic effect of elements on bone turnover. Biomarkers of bone formation are products that active osteoblasts express at different stages of their maturation or bone enzymes. Biomarkers that are widely measured in serum or plasma include bone-specific alkaline phosphatase (BSAP), and osteocalcin, which were measured in the present study (10).

Bone-specific alkaline phosphatase is a biomarker involved in bone formation. Alkaline phosphatase (ALP, ALKP, ALPase, Alk Phos) (EC3.1.3.1) is a hydrolase enzyme (associated with cell membranes) that is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. Alkaline phosphatase is produced by osteoblasts (11).

Osteocalcin is a non-collagenous protein made by osteoblasts. Osteocalcin is found in bone and has a metabolic role, and is prosteoblastic. Osteocalcin is involved in bone mineralization and calcium homeostasis. Serum osteocalcin levels are determined by bone formation and the number of osteoblasts. Serum osteocalcin levels are determined by histomorphometry and calcium analysis and are introduced as a biomarker of bone formation. Osteocalcin is one of a few proteins that are specific to the skeletal system (12). Osteocalcin and alkaline phosphatase are good biomarkers for the study of osteoblast activity. On the other hand, zinc is essential for osteoblast activity, collagen synthesis, and alkaline phosphatase activity (13).

Considering that the changes in bone during space exploration in astronauts are one of the most important issues in astronomy, this study was designed to determine the changes in bone biomarkers with and without zinc sulfate in an experimental model of space exploration.

Due to the high cost of space exploration, efforts have been made to artificially create weightlessness on Earth. One of these methods, hanging a rat by its tail, is widely used as a laboratory model of space exploration. This technique simulates the effects of weightlessness on the bones and muscles of the animal's hind legs. It also allows the blood flow to be simulated, like a human being in a state of weightlessness, in which the blood flow tends to shift towards the animal's head and chest (14).

Materials And Methods

Type of study and time of study

This experimental study was performed in the animal husbandry of Iran University of Medical Sciences with the participation of the Faculty of Aerospace and submarine Medicine of AJA University of Medical Sciences during 2021.

Sample size

Based on animal studies, the sample size was 14 rats (7 rats in each group).

Study population

Fourteen 6-week-old male rats were obtained from the breeding colony at Iran University of Medical Sciences, Tehran, Iran. These rats were randomly divided into two groups. Group A included 7 rats as the intervention group; group B included 7 rats as the control group.

Procedure

The two groups of 7 rats were first housed for one week at the same temperature (23±2 °C) and humidity (60±10) with a 12 hr light-12 hour dark system and maintained on the same diet.

Then, rats from each group were individually housed in cages designed so that the rat’s tail was attached to the roof of the cage and the rat was suspended by the tail (hindlimb suspension). These conditions, remove pressure from the back legs of the rat. The animal could move in the cage using its front legs. This technique simulates conditions similar to microgravity during space exploration (14).

Both groups were given a normal diet. Rats in group A received 227 mg/l of oral zinc sulfate (prepared by Bahansar Company) in their drinking water. This does is based on previous study (15). Rats in group control received placebo. The study period lasted 30 days.

A blood sample was taken at the beginning of the study and on day 30 to check for changes in bone biomarkers (alkaline phosphatase and osteocalcin) and the amount of circulating zinc. The samples were then transferred to the laboratory, the serum was separated, frozen, and kept at -18 °C until the end of the study. For all samples, bone biomarkers of alkaline phosphatase, osteocalcin, and serum zinc levels were measured and compared using kits purchased from Eastbiopharm.

Hindlimb suspension

The technique of hindlimb suspension has been approved by NASA (14) as a simulation of the conditions experienced in space. The main cause of osteoporosis in space exploration is the lack of gravity and, consequently the lack of sufficient pressure on the weight-bearing bones. Hindlimb suspension prevents the lower limbs from touching the ground and bearing weight, inducing osteoporosis (as relied on by NASA's Hindlimb Suspension model).

To facilitate feeding and the isolation of the rat from urine and feces, the body of the cages was designed similar to that of metabolic cages. Because rats have a high ability to release their tails, it was thought that the tail should come out of the cage. For this reason, a hole was made in the roof of the cage (in its central part) to exit the tail. The size of the cage was designed so that the rats could easily access the water and food tank despite hanging from the tail area. At the exit of the tail from the roof of the cage, a plastic plate was added to eliminate the minimum contact between the rat and the tail and to minimize the possibility of chewing the glue and releasing the tail.

Placement of rats in the suspension cage

The rats were gently placed inside the special chamber. Their tails were cleaned with alcohol to remove the dead and dirty tissues. A disposable plate made from herbal ingredients was pierced through the middle and the tail was passed through it. Herbal ingredients were chosen to prevent contact dermatitis resulting from chemicals and damage to the rat's tail. The tail was then passed through the middle hole of the cage lid plate. The piston of a 2 cc syringe was then cut in half with a small chainsaw. A layer of sterile gauze was wrapped around the rat's tail and then the rat's tail was placed inside the cut syringe and sealed with glue. The bandages were loose enough to allow blood to circulate. However, because the tail plays an important role in regulating rat body temperature, only areas of the tail that were attached to the syringe were wrapped by sterile gauze. The rat was then placed in a cage. The bulge at the end of the syringe was trapped in the lid of the cage, keeping the rat hanging. The rat's body was made at a 45-degree angle to the cage floor, so the hind legs did not come into contact with the cage floor net. The rat was placed in such a way that it had easy access to water and food.

Ethical principles

The study was conducted by the Declaration of Helsinki and the guidelines of the Animal Welfare Association of Canada. Institutional Review Board approval (code: 92108- AJA University of Medical Sciences) was obtained.

Statistical analysis

The continuous variables were expressed as the mean±SD, and the categorical variables were presented as a percentage. Chi-square and independent t-test were used to compare data between the two groups and a repeatd measures test have been used for within groups' comparisons. All statistical analyses were

performed with SPSS (version 16.0, SPSS Inc, Chicago, IL, USA). A “P-value” less than 0.05 was considered significant.

Results

Weight of rats

Fourteen male Wistar rats were divided into two groups of 7; intervention group (microgravity simulation model with oral zinc supplementation - Zn) and control group (microgravity simulation model with placebo). The average weight of the rats in the Zn group was 250 ± 12 g. The average weight of the control group was 245 ± 8 g. There was no significant difference between the groups (p = 0.38).

Zinc (Zn)

The mean serum zinc level in the control group was 91.5 ± 4.4 mcg/dl on day 0 and 90.6 ± 3.5 mcg/dl on day 30 and there was no significant difference in serum zinc levels between the two samples (p = 0.805). The mean serum zinc level in the Zn group was 90.4 ± 4.45 mcg/dl on day 0 and 229 ± 8.68 mcg/dl on day 30 and there was a significant difference between the two samples (p = 0.001) (Table 1).

The baseline serum zinc levels in rats in the two groups were similar. After 30 days, serum zinc levels in rats that received oral zinc sulfate was significantly higher than the control group that received a placebo (Table 1).

Table-1. Comparison of serum zinc levels in rats in two groups (zinc and control) in stages of the study (first, day 30)

	Zinc Sulfate Supplemet	Placebo	P value
Baseline	90.4 ± 5.4	91.5 ± 4.4	0.67
Day 30	229 ± 8.7	90.6 ± 3.5	0.0001
P value	0.0001	0.68	

Alkaline phosphatase

A significant increase in alkaline phosphatase was recorded during 30 days in the control group (p = 0.002) and intervention group (p = 0.002). This increase in alkaline phosphatase in the intervention group on days 30 is greater than that in the control group, which is statistically significant ($p \leq 0.05$; Table 2).

Table – 2. Comparison of mean alkaline phosphatase biomarker values in both intervention groups (microgravity simulation model with oral zinc supplementation) and control group (microgravity simulation model with placebo) between days 0 and 30

	Placebo	Zinc Sulfate Supplemet	P value
Baseline	130.4 ± 9.2	131.6 ± 10.3	0.82
Day 30	209.3 ± 2	436.6 ± 90.7	0.0001
P value	0.0001	0.0001	

Osteocalcin

Osteocalcin level decreased significantly in the intervention (p = 0.001) and control (p = 0.001) groups during 30 days. No significant difference was observed between the two groups in baseline and day 30 (Table 3).

Table – 3. Comparison of mean Osteocalcin biomarker values in both intervention groups (microgravity simulation model with oral zinc supplementation) and control group (microgravity simulation model with placebo) between days 0 and 30

	Placebo	Zinc Sulfate Supplemet	P-value
Baseline	58.4 ± 2.9	58.3 ± 1.9	0.94
Day 30	45.6 ± 1.9	44.7 ± 4.9	0.66
P-value	0.0001	0.0001	

Discusscion

In the present study, the effect of oral supplementation of zinc and microgravity on changes in the two factors affecting bone formation, namely alkaline phosphatase and osteocalcin in blood serum during 30 days (in microgravity conditions using a simulated model of space exploration or Tail Suspension) was investigated. Weightlessness caused a significant decrease in osteocalcin in both the presence and absence of Zn supplement. In contrast, no effect was recorded in the zinc-receiving group, and zinc administration did not have an effect on osteocalcin improvement. While weightlessness caused a significant increase in alkaline phosphatase in both groups, the addition of zinc significantly increased the phosphatase levels above that of controls.

Bone loss during space exploration is about 1–2% per month (2, 3). Bone loss in space is more common in weight-bearing bones of the lower limbs and spine. People who spend six months in space, lose about 20 percent of their lower limb bone mass. In addition, after returning to Earth, bone loss continues for months. Loss of bone to this extent leads to a significant increase in the risk of fracture by about 5 times more than expected in normal bone on Earth (16, 17).

Consistent with the current finding, Grano et al (2005) showed that microgravity induced by hindlimb suspension for 35 days reduces osteocalcin activity and increases alkaline phosphatase activity (18). In 1994, Backup et al. found the level of osteocalcin in the bones decreased after 10 days of space flight (19). Patterson-Buckendahl et al. examined the effects of microgravity on osteocalcin in mice in Spacelab, and found that osteocalcin levels decreased during the first week of suspension and returned toward control values after 15 days (20).

The effectiveness of zinc in increasing alkaline phosphatase activity was confirmed. Yamaguchi et al. (2008) showed that zinc stimulated bone metabolism in rats, increased bone protein synthesis, and bone formation in cultured tissues by increasing enzyme activity such as alkaline phosphatase (21). Lowe et al. (2002) showed that zinc is one of the components involved in the synthesis of alkaline phosphatase and is essential for its activity (22). Sibonga et al. (2007) observed an increase in osteoblast-specific proteins, alkaline phosphatase and osteocalcin, after returning to Earth's gravity (23). Park et al (2013) found that zinc inhibits osteoclastogenesis in rats by inhibiting the signaling pathway of Ca^{2+}-Calcineurin-NFATc1 in bone marrow-derived monocytes. They suggested that zinc could be a good candidate for the treatment of osteoporosis due to the activation of NFATc1 in osteoclasts (24). Seveal studies have shown that dietary zinc is an effective measure in preventing bone loss. Chou et al. (2013) found that biomimetic zinc was effective in preventing and treating osteoporosis in a rat model (25). Sun et al., (2011) examined the found that dietary zinc was effective on bone growth, metabolism, and IGF-I gene expression and alkaline phosphatase (26). Seo et al. (2010) reported that zinc production increases osteogenic activity by stimulating cell proliferation, alkaline phosphatase activity and collagen synthesis in osteoblast cells in culture (27).

Conclusion

A microgravity simulation model for space exploration increased alkaline phosphatase and decresed osteocalsine in rats. Adding zinc sulphate to diet was effective in increasing the amount of alkaline phosphatase but failed to show a significant effect on osteocalcin reduction.

Abbreviations

Zinc: Zn;

Bone-specific alkaline phosphatase: BSAP;

Human Research Roadmap: HRR;

National Aeronautics and Space Administration: NASA;

Alkaline phosphatase: ALP, ALKP, ALPase, Alk Phos.

Declarations

Acknowledgment

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Authors' contributions

RE, AK were responsible for study concept and design. RE, AK led data collection. JS, RH were responsible for the analysis and interpretation of data. RE wrote the first draft. JS, RH contributed to the writing of the second and third draft. JS, RH provided comments on initial drafts and coordinated the final draft. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted by the Declaration of Helsinki and the guidelines of the Animal Welfare Association of Canada. Institutional Review Board approval (code: 92108- AJA University of Medical Sciences) was obtained.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

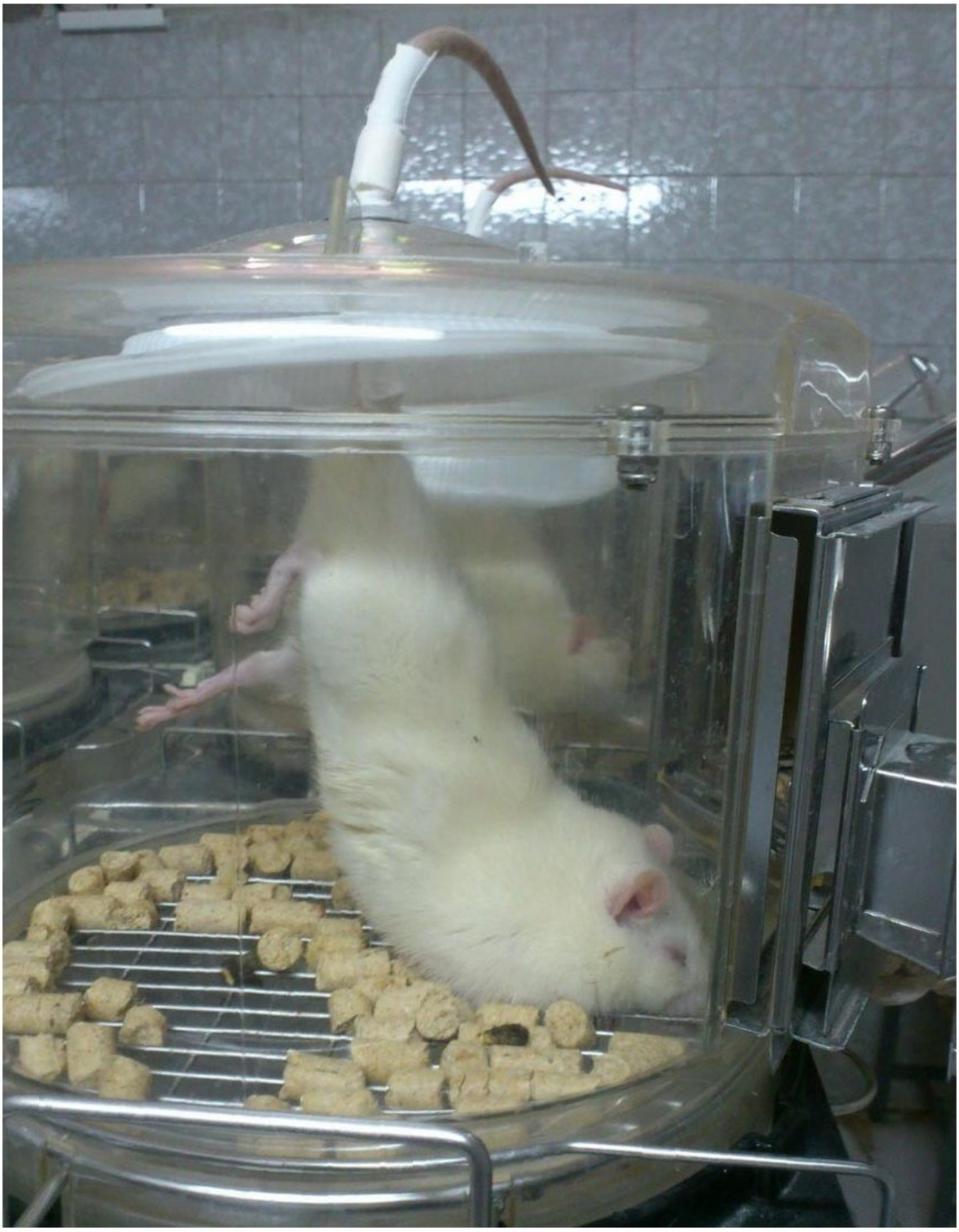

Figure 1

Rat placement conditions in the designed and built model